Role of Folic Acid in Birth Defects Prevention Epidemiologic Perspectives

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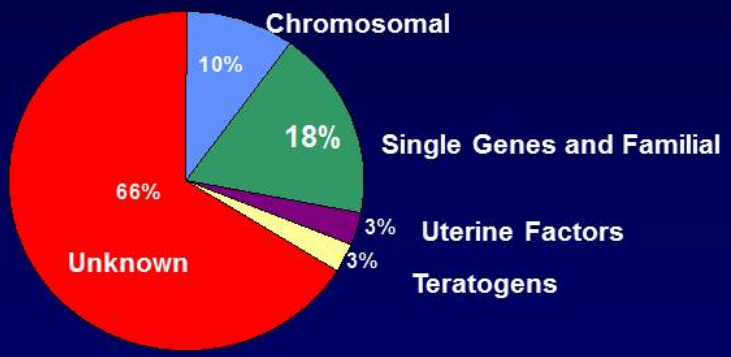
Population Burden-Birth Defects

- 1 in 33 babies born with birth defects (6% in world)
- >100,000/year in US
- 1 in 12 for preterm babies
- ~20% of infant deaths
- ~ 60% of neonatal deaths >1499 gm

Early

Most occur within the first 3 months of pregnancy

The Unknown Fraction



Nelson & Holmes, *N Engl J Med* 320:19-23, 1989

ARCHIVES OF DISEASE IN CHILDHOOD
The Journal of the Royal College of Paediatrics and Child Health

Vitamin deficiencies and neural tube defects

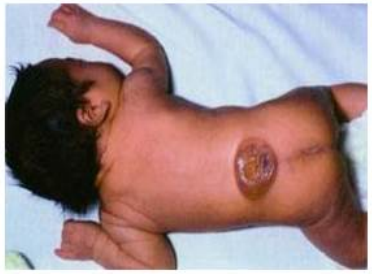
R.W. SMITHELLS, S. SHEPPARD, and C.J. SCHORAH

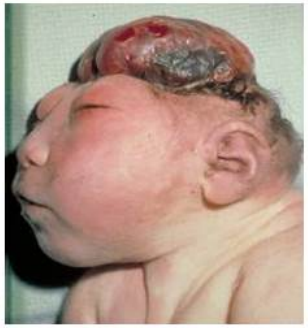
1976

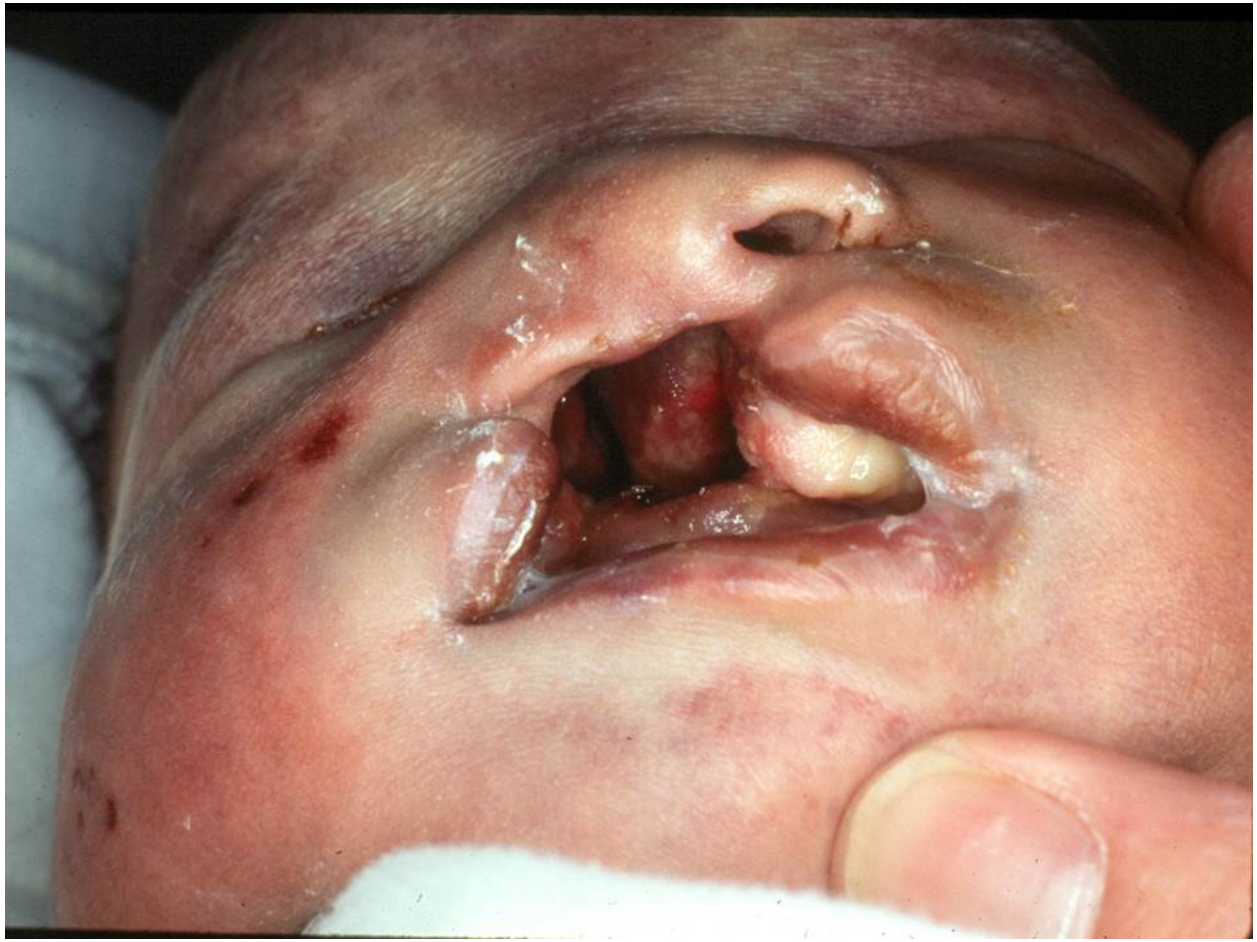
"These results must be interpreted with caution. If vitamin deficiency is a factor in the genesis of CNS defects, appropriate vitamin supplementation might make a contribution to primary prevention."


NATURE'S BOUNTY
MAXIMUM STRENGTH
FOLIC ACID
800 mcg
250 TABLETS
B

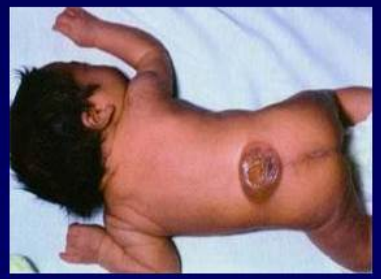
Neural Tube Defects



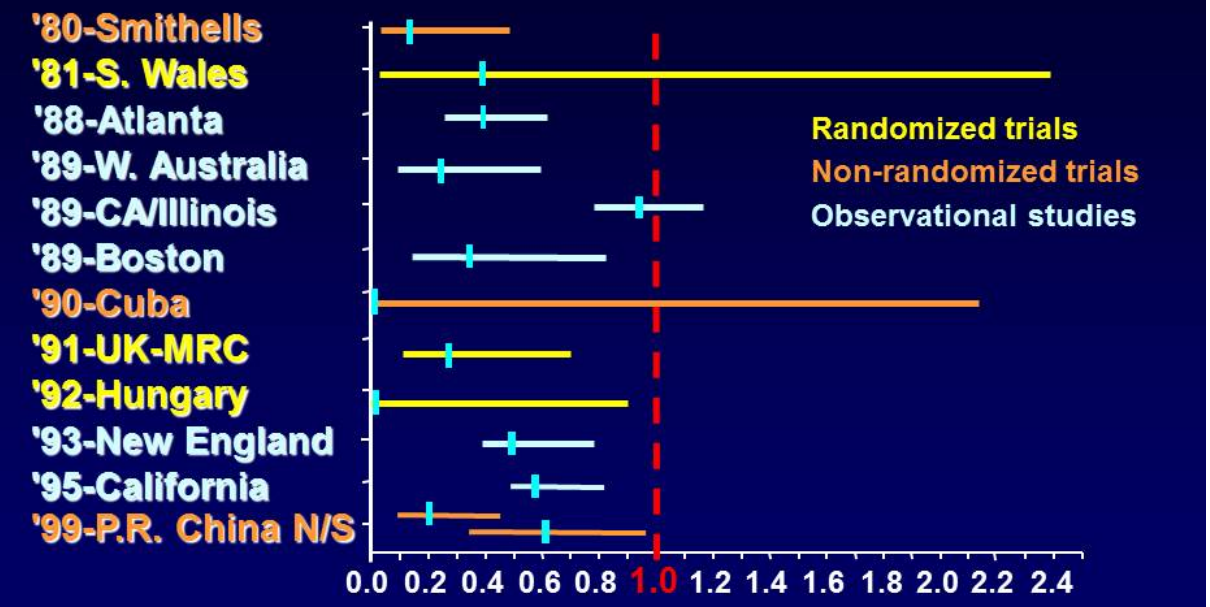




What is the epidemiologic evidence?

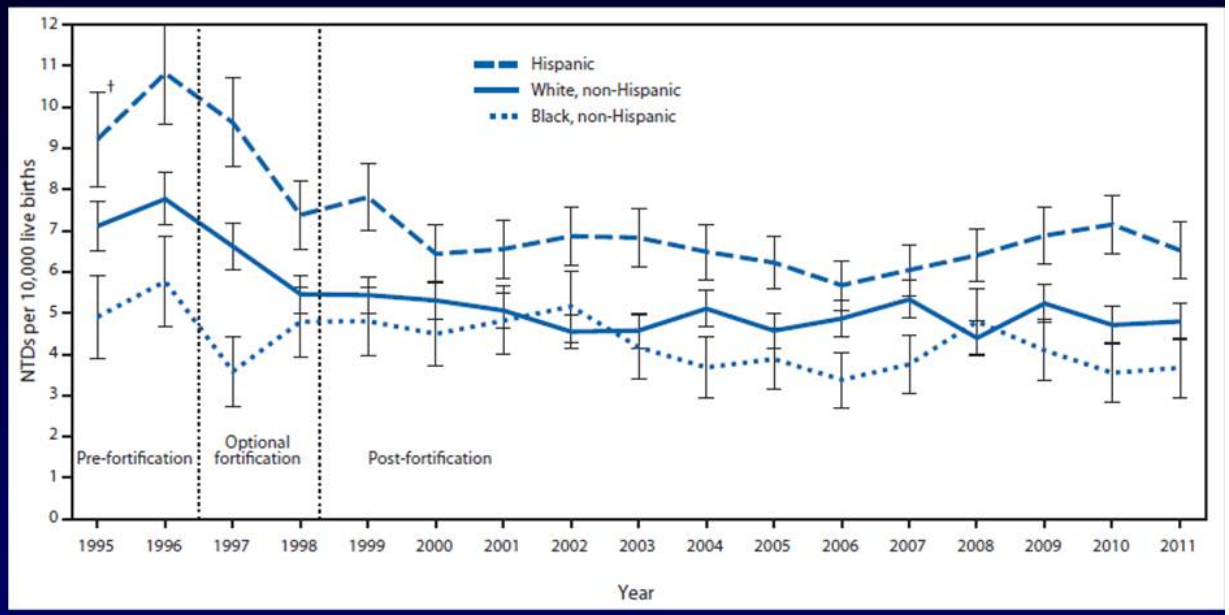


Effect of multivitamins containing folic acid on the risk for neural tube defects, 1980-1999



Reduction in Neural Tube Defects After Fortification

↓15-50% across many registries & countries



MMWR Jan 2015

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics

Wheat Flour Fortification With Folic Acid: Changes in Neural Tube Defects Rates in Chile

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Manuscript Received: 13 September 2011; Manuscript Accepted: 26 March 2012

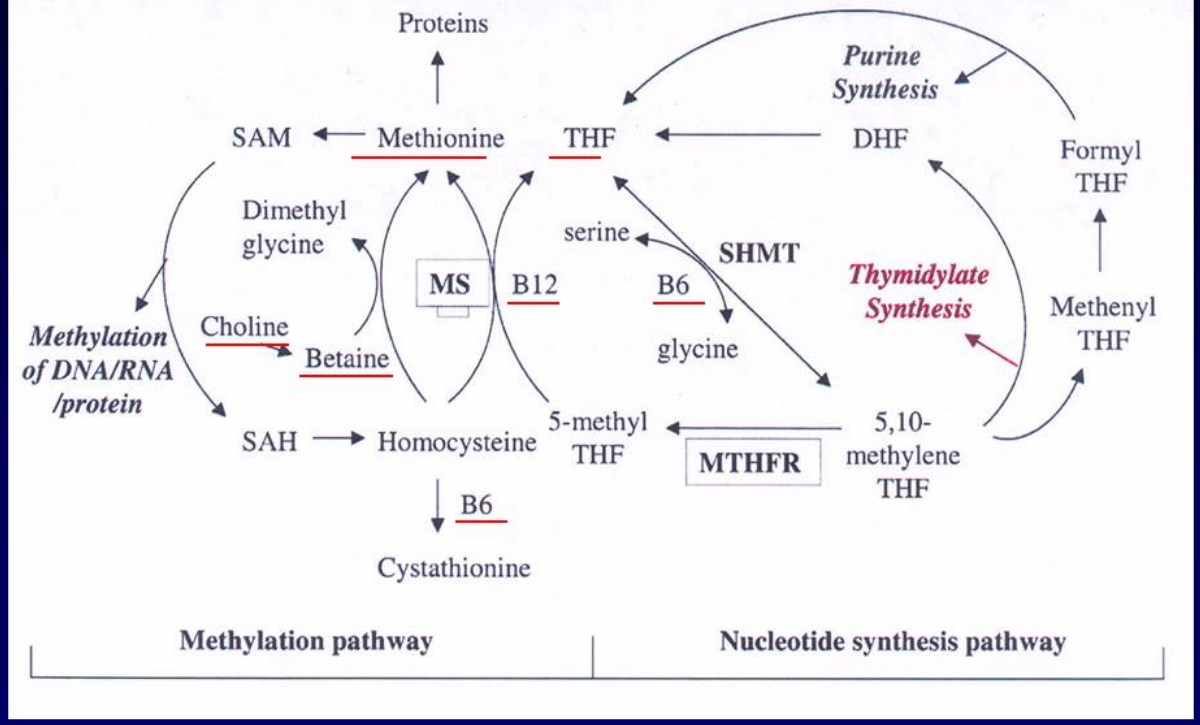
In January 2000, Chilean Ministry of Health mandated the addition of folic acid (FA) to wheat flour in order to reduce the risk of neural tube defects (NTDs). This policy resulted in significant increases in serum and red cell folate in women of fertile age 1 year after fortification. To evaluate the effect of wheat flour fortification on the prevalence of NTDs in Chile we designed a prospective hospital-based surveillance program to monitor the frequency of NTDs in all births (live and stillbirths) with birth weight ≥500 g at the nine public maternity hospitals of Santiago, Chile from 1999 to 2009. During the pre-fortification period (1999–2000) the NTD rate was 17.1/10,000 births in a total of 120,566 newborns. During the post-fortification period (2001–2009) the NTD rate decreased to 8.6/10,000 births in a total of 489,915 newborns, which translates into a rate reduction of 50% (RR: 0.5; 95% CI: 0.42–0.59) for all NTDs. The rate reduction by type of NTD studied was: 50% in anencephaly (RR: 0.5; 95% CI: 0.38–0.67), 42% in cephalocele (RR: 0.58; 95% CI: 0.37–0.89), and 52% in spina bifida (RR: 0.48; 95% CI: 0.38–0.6). Rates showed significant reduction both in stillbirths and live

How to Cite this Article:
Cortés F, Mellado C, Pardo RA, Villarroel LA, Hertrampf E. 2012. Wheat flour fortification with folic acid: Changes in neural tube defects rates in Chile.
Am J Med Genet Part A 158A:1885–1890.

Dudas, 1992]. Recommendations from North America [Centers f
Disease Control and Prevention (CDC) 1992], Europe [Scientif
Committee on Human Nutrition, 1993], and FAO/WHO (FA
WHO expert consultation, 2002) are in agreement that all wom
in fertile age should consume 400 μg of FA daily to reduce their ri
of having a pregnancy affected by NTDs.

FA supplementation and food fortification are being used
increase FA intake by women in fertile age. Prevention polici
focused on the use of dietary supplements have not been effective

How does **folic acid** alter risk of NTDs?


Proteins
Purine Synthesis
SAM
Methionine
THF
DHF
Formyl THF
Dimethyl glycine
serine
SHMT
MS
B12
B6
Thymidylate Synthesis
Methenyl THF
Methylation of DNA/RNA /protein
Choline
Betaine
glycine
SAH
Homocysteine
5-methyl THF
MTHFR
5,10-methylene THF
B6
Cystathionine
Methylation pathway
Nucleotide synthesis pathway

5,10-*MTHFR*

Gene-only effects

meta-analysis, odds ratio=1.8

Botto & Yang 2000

MTHFR polymorphism and Spina Bifida risk

Maternal vitamin use (folic acid

TT vs CC

Odds ratio = 1.2 (0.4-4.0)

MTHFR polymorphism and Spina Bifida risk

Maternal vitamin use (folic acid	No vitamin use (folic acid)
TT vs CC	
Odds ratio = 1.2 (0.4-4.0)	Odds ratio = 1.6 (0.8-3.1)

Neural Tube Defects and Folate Pathway Genes: Family-Based Association Tests of Gene–Gene and Gene–Environment Interactions

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BACKGROUND: Folate metabolism pathway genes have been examined for association with neural tube defects (NTDs) because folic acid supplementation reduces the risk of this debilitating birth defect. Most studies addressed these genes individually, often with different populations providing conflicting results.

OBJECTIVES: Our study evaluates several folate pathway genes for association with human NTDs, incorporating an environmental cofactor: maternal folate supplementation.

METHODS: In 304 Caucasian American NTD families with myelomeningocele or anencephaly, we examined 28 polymorphisms in 11 genes: folate receptor 1, folate receptor 2, solute carrier family 19 member 1, transcobalamin II, methylenetetrahydrofolate dehydrogenase 1, serine hydroxymethyltransferase 1, 5,10-methylenetetrahydrofolate reductase (*MTHFR*), 5-methyltetrahydrofolate-homocysteine methyltransferase, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, betaine-homocysteine methyltransferase (*BHMT*), and cystathionine-beta-synthase.

RESULTS: Only single nucleotide polymorphisms (SNPs) in *BHMT* were significantly associated in the overall data set; this significance was strongest when mothers took folate-containing nutritional supplements before conception. The *BHMT* SNP rs3733890 was more significant when the data were stratified by preferential transmission of the *MTHFR* rs1801133 thermolabile T allele from parent to offspring. Other SNPs in folate pathway genes were marginally significant in some analyses when stratified by maternal supplementation, *MTHFR*, or *BHMT* allele transmission.

CONCLUSIONS: *BHMT* rs3733890 is significantly associated in our data set, whereas *MTHFR* rs1801133 is not a major risk factor. Further investigation of folate and methionine cycle genes will require extensive SNP genotyping and/or resequencing to identify novel variants, inclusion of environmental factors, and investigation of gene–gene interactions in large data sets.

KEY WORDS: folate, folic acid supplementation, genetic association, neural tube defects. *Environ Health Perspect* 114:1547–1552 (2006). doi:10.1289/ehp.9166 available via *http://dx.doi.org/* [Online 15 June 2006]

(*SLC19A1*; GenBank accession no. U15939), also known as reduced folate carrier protein 1. Transcobalamin II (*TCN2*; GenBank accession no. NM_000355) imports vitamin B_{12}, cobalamin, a cofactor for another folate enzyme, 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*; GenBank accession no. NM_000254). The reactions within the folate metabolism cycle can be very complex, with methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*; GenBank accession no. J04031), serine hydroxymethyltransferase 1 (*SHMT1*; GenBank accession no. NM_004169), and 5,10-methylenetetrahydrofolate reductase (*MTHFR*; GenBank accession no. NM_005957) being widely studied in the NTD literature.

MTHFR rs1801133 is the most frequently investigated polymorphism in NTDs with conflicting results in different populations: Dutch and Irish populations associate the TT allele with risk (Shields et al. 1999; van der Put et al. 1995), whereas a protective effect is seen in Italians (De Marco et al. 2002) and other populations have no evidence of association (Gonzalez-Herrera et al. 2002; Revilla et al.

Birth Defects Research (Part A) 85:285–294 (2009)

Review Article

The Search for Genetic Polymorphisms in the Homocysteine/Folate Pathway That Contribute to the Etiology of Human Neural Tube Defects

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Received 13 October 2008; Revised 1 December 2008; Accepted 3 December 2008

In this paper, we trace the history of current research into the genetic and biochemical mechanisms that underlie folate-preventable neural tube defects (NTDs). The inspired suggestion by Smithells that common vitamins might prevent NTDs ignited a decade of biochemical investigations—first exploring the nutritional and metabolic factors related to NTDs, then onto the hunt for NTD genes. Although NTDs were known to have a strong genetic component, the concept of common genetic variance being linked to disease risk was relatively novel in 1995, when the first folate-related polymorphism associated with NTDs was discovered. The realization that more genes must be involved started a rush to find polymorphic needles in genetic haystacks. Early efforts entailed the intellectually challenging and time-consuming task of identifying and analyzing candidate single nucleotide polymorphisms (SNPs) in folate pathway genes. Luckily, human genome research has developed rapidly, and the search for the genetic factors that contribute to the etiology of human NTDs has evolved to mirror the increased level of knowledge and data available on the human genome. Large-scale candidate gene analysis and genome-wide association studies are now readily available. With the technical hurdles removed, the remaining challenge is to gather a sample large enough to uncover the polymorphisms that contribute to NTD risk. In some respects the real work is beginning. Although moving forward is exciting, it is humbling that the most important result—prevention of NTDs by maternal folic

14 genes – 118 SNPs

- *BHMT* 8
- *BHMT2* 7
- *CBS* 9
- *DHFR* 9
- *FOLR1* 3
- *FOLR2* 3
- *MTHFD1* 10
- *MTHFD2* 8
- *MTHFR* 13
- *MTR* 21
- *MTRR* 13
- *NOS3* 3
- *RFC1* 6
- *TYMS* 5

Conclusion

……observations do not implicate a particular {polymorphism} folate transport or metabolism gene to be strongly associated with risks for spina bifida …….

OPEN ACCESS Freely available online

PLoS one

A Genetic Signature of Spina Bifida Risk from Pathway-Informed Comprehensive Gene-Variant Analysis

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1 Department of Molecular and Cellular Biology, California Institute for Quantitative Biosciences, University of California, Berkeley, California, United States of America, **2** Department of Epidemiology and Biostatistics and Institute of Human Genetics, University of California San Francisco, San Francisco, California, United States of America, **3** Children's Hospital Oakland Research Institute, Oakland, California, United States of America, **4** VitaPath Genetics, Inc., Foster City, California, United States of America, **5** Department of Energy, Joint Genome Institute, Walnut Creek, California, United States of America, **6** Department of Pediatrics, Stanford University School of Medicine, Stanford, California, United States of America

Abstract

Despite compelling epidemiological evidence that folic acid supplements reduce the frequency of neural tube defects (NTDs) in newborns, common variant association studies with folate metabolism genes have failed to explain the majority of NTD risk. The contribution of rare alleles as well as genetic interactions within the folate pathway have not been extensively studied in the context of NTDs. Thus, we sequenced the exons in 31 folate-related genes in a 480-member NTD case-control population to identify the full spectrum of allelic variation and determine whether rare alleles or obvious genetic interactions within this pathway affect NTD risk. We constructed a pathway model, predetermined independent of the data, which grouped genes into coherent sets reflecting the distinct metabolic compartments in the folate/one-carbon pathway (purine synthesis, pyrimidine synthesis, and homocysteine recycling to methionine). By integrating multiple variants based on these groupings, we uncovered two provocative, complex genetic risk signatures. Interestingly, these signatures differed by race/ethnicity: a Hispanic risk profile pointed to alterations in purine biosynthesis, whereas that in non-Hispanic whites implicated homocysteine metabolism. In contrast, parallel analyses that focused on individual alleles, or individual genes, as the units by which to assign risk revealed no compelling associations. These results suggest that the ability to layer pathway relationships onto clinical variant data can be uniquely informative for identifying genetic risk as well as for generating mechanistic hypotheses. Furthermore, the identification of ethnic-specific risk signatures for spina bifida resonated with epidemiological data suggesting that the underlying pathogenesis may differ between Hispanic and non-Hispanic groups.

Multiple alleles

Mutation loads

Higher in controls!

Nature knows what she is doing, and does it, even when we cannot find out.

- Sir Arthur Stanley Eddington

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Autoantibodies against Folate Receptors in Women with a Pregnancy Complicated by a Neural-Tube Defect

Sheldon P. Rothenberg, M.D., Maria P. da Costa, M.D., Jeffrey M. Sequeira, M.S., Joan Cracco, M.D., Jadyn L. Roberts, M.D., Jeremy Weedon, Ph.D., and Edward V. Quadros, Ph.D.

From the Departments of Medicine (S.P.R., M.P.C., J.M.S., E.V.Q.) and Obstetrics and Gynecology (J.L.R.), the Division of Pediatric Neurology (J.C.), and the Scientific Computing Center (J.W.), State University of New York Downstate Medical Center, Brooklyn. Address reprint requests to Dr. Rothenberg at SUNY Downstate Medical Center, 450 Clarkson Ave., Box 20, Brooklyn, NY 11203, or at srothenberg@downstate.edu.

N Engl J Med 2004;350:134-42.

ABSTRACT

BACKGROUND

In the absence of clinical folate deficiency, periconceptional supplementation with folic acid reduces a woman's risk of having an infant with a neural-tube defect. Since antiserum to folate receptors induces embryo resorption and malformations in rats, we hypothesized that autoantibodies against folate receptors in women may be associated with pregnancy complicated by a neural-tube defect.

METHODS

Serum from 12 women who were or had been pregnant with a fetus with a neural-tube defect and from 24 control women (20 with current or prior normal pregnancies and 4 who were nulligravid) was analyzed for autoantibodies by incubation with human placental folate receptors radiolabeled with [^{3}H]folic acid. The properties of these autoantibodies were characterized by incubating serum and the autoantibodies isolated from serum with placental membranes, ED27 cells, and KB cells, which express the folate receptors.

RESULTS

Serum from 9 of 12 women with a current or previous affected pregnancy (index subjects) and 2 of 20 control subjects contained autoantibodies against folate receptors ($P<0.001$). The autoantibodies blocked the binding of [^{3}H]folic acid to folate receptors on placental membranes and on ED27 and KB cells incubated at 4°C and blocked the uptake of [^{3}H]folic acid by KB cells when incubated at 37°C.

CONCLUSIONS

Serum from women with a pregnancy complicated by a neural-tube defect contains autoantibodies that bind to folate receptors and can block the cellular uptake of folate. Further study is warranted to assess whether the observed association between maternal autoantibodies against folate receptors and neural-tube defects reflects a causal relation.

Folic Acid During Embryogenesis?

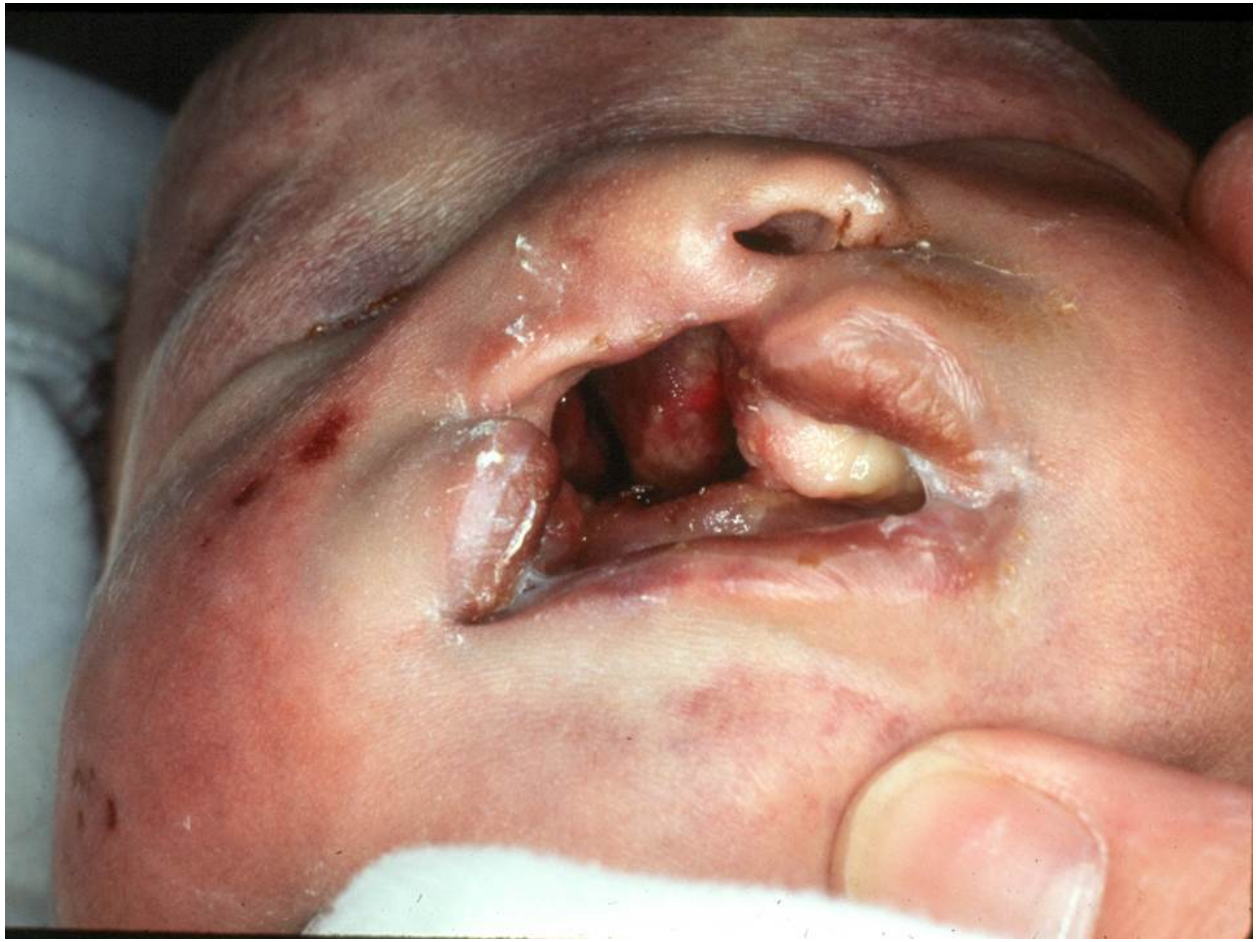
Women produce autoantibodies to folate receptors preventing binding and transport of folic acid to cellular components during embryonic development.

Supplemental folates compete with blocking antibodies and restore cellular folate concentrations.

Folate AutoAntibody Titers - Mean

	Controls	NTDs	*p*-value
FBP (IgM)	50.4	66.1	0.04
FR (IgG)	5.7	12.5	0.02
FR (IgM)	59.0	79.5	<0.001

Cabrera et al. 2008



Nitric Oxide Synthase

- *NOS3* variants influence (raise) homocysteine concentrations
- smoking compromises *NOS3* activity
- folate intake influences (lowers) homocysteine concentrations
- is clefting risk from *NOS3* variants modified by smoking and further modified by vitamin intake (folic acid)?

NOS3 **A922G** genotypes, maternal smoking, maternal vitamin use, and Cleft lip/palate risks

Genotype	Smoking	Vitamin Use	Odds Ratio	95% CI
Variant	Yes	No	4.6	2.0-11.0
Variant	Yes	Yes	1.6	0.9-2.8
Wildtype	Yes	No	1.4	0.5-4.0
Wildtype	Yes	Yes	1.4	0.8-2.6
Wildtype	No	Yes	Ref	-----

Shaw et al. 2004

The FASEB Journal • Life Sciences Forum

Folic acid in early pregnancy: a public health success story

Sarah G. Običan,* Richard H. Finnell,† James L. Mills,‡ Gary M. Shaw,§ and Anthony R. Scialli*,‖,1

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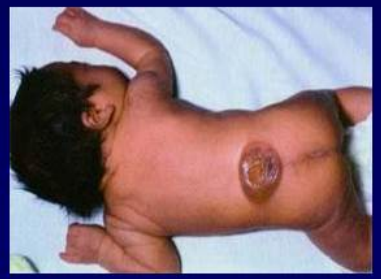
ABSTRACT **Folate is a water-soluble B vitamin that must be obtained in the diet or through supplementation. For >50 yr, it has been known that folate plays an integral role in embryonic development. In mice, inactivation of genes in the folate pathway results in malformations of the neural tube, heart, and craniofacial structures. It has been shown that diets and blood levels of women who had a fetus with a neural tube defect are low for several micronutrients, particularly folate. Periconceptional use of folic acid containing supplements decreased recurrent neural tube defects in the offspring of women with a previously affected child and the occurrence of a neural tube defect and possibly other birth defects in the offspring of women with no prior history. Based on these findings, the U.S. Public Health Service recommended that all women at risk take folic acid supplements, but many did not. Mandatory food fortification programs were introduced in numerous countries, including the United States, to**

prevalence of antenatal diagnostic testing. Neural tube defects result from the incomplete closure of the neural tube during the fourth week of gestation (2). The most common neural tube defects are spina bifida, due to incomplete closure of the caudal neural tube, and anencephaly, due to incomplete closure of the rostral end of the neural tube. These malformations are fatal or result in significant lifelong disability.

For >50 yr, it has been known that folate plays an integral role in embryonic development. The investigation into the role of folate in neural tube defects and the use of folic acid supplementation to prevent these and perhaps other malformations has been an example of how scientists in diverse fields have worked together to favorably affect the public health. The Teratology Society is proud that many of these scientists are Society members, and many of the discussions leading to this major public health contribution took place at annual

Alternative interpretations?

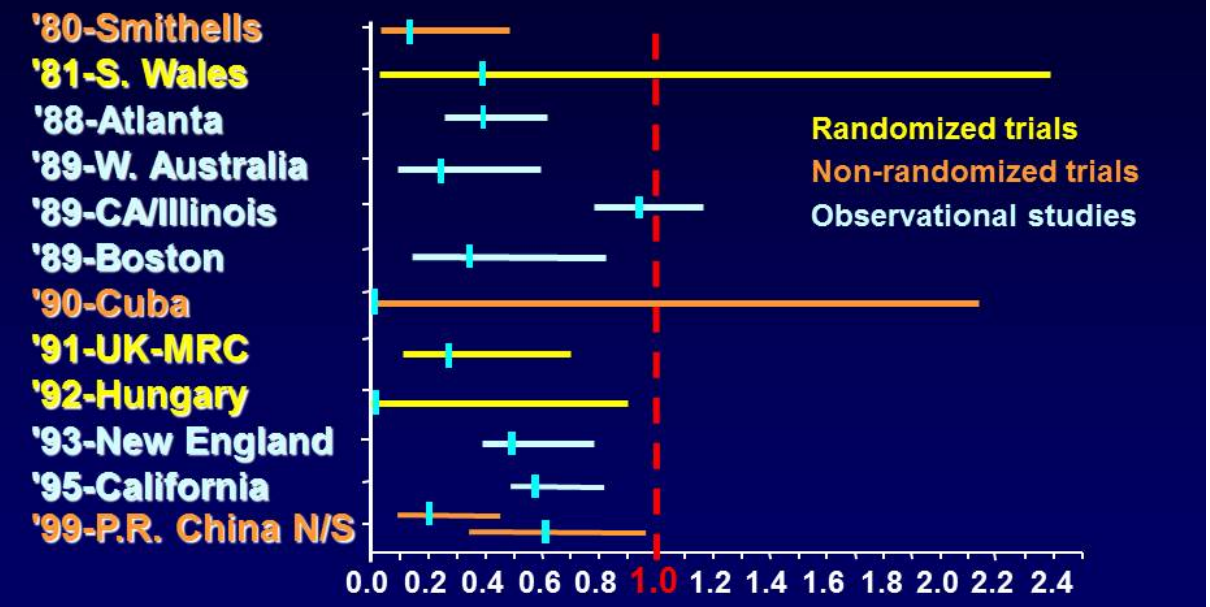
Do the folic acid results fit our expectation?



Detracting Evidence or Clues

- Nearly all studies included other vitamins

Effect of multivitamins containing folic acid on the risk for neural tube defects, 1980-1999



MRC Randomized Trial

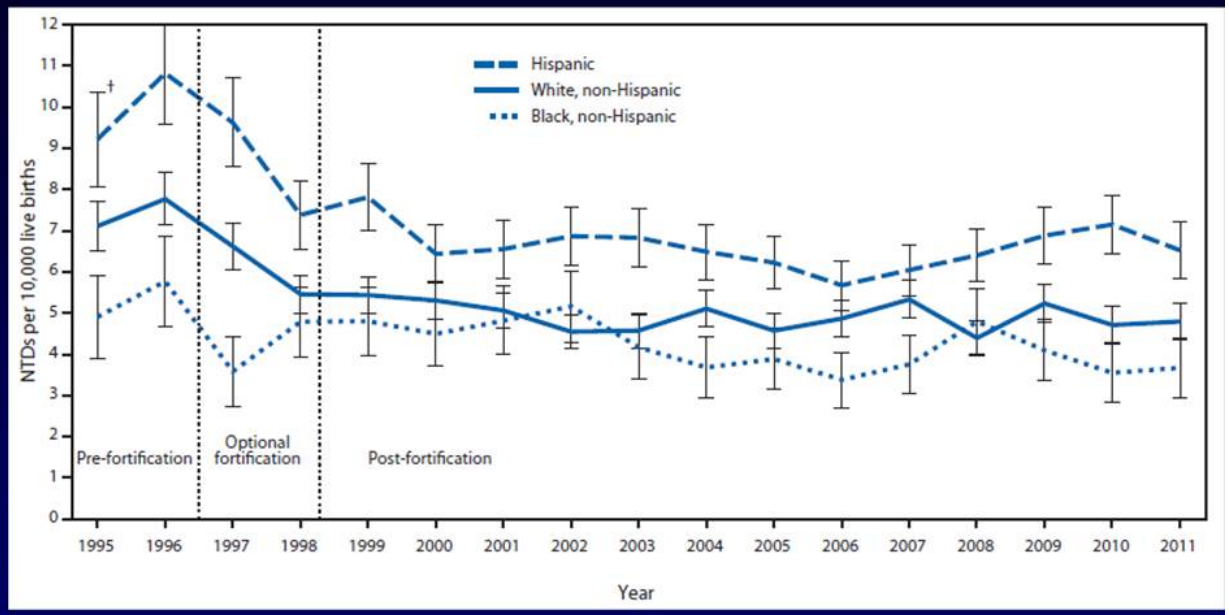
	Relative Risk	95% CI
Folic Acid only vs. Placebo	**0.15**	0.05-0.70
Other Vitamins vs. Placebo	**0.60**	0.26-1.50

Detracting Evidence or Clues

- Nearly all studies included other vitamins
- **Inconsistency across population subgroups**

Use of folic acid in periconceptional period among Hispanics and risk for NTDs

	Odds ratio
California	0.96
Texas	1.12

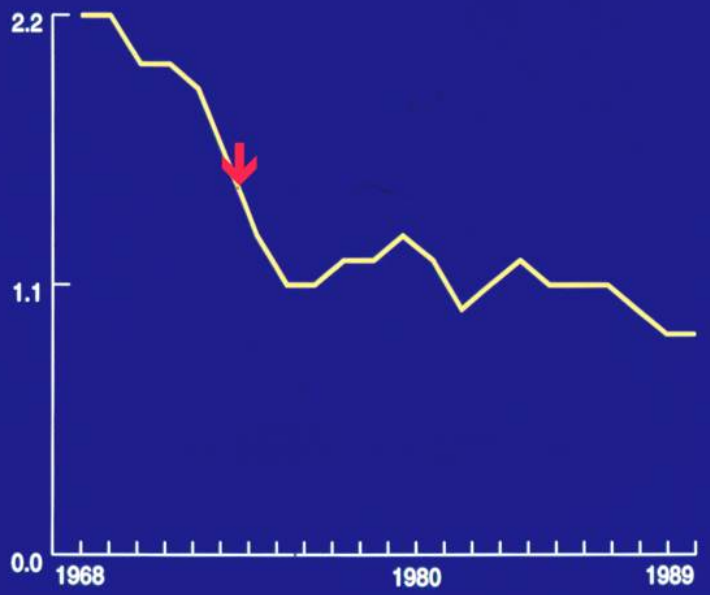


MMWR Jan 2015

Detracting Evidence or Clues

- Nearly all studies included other vitamins
- Inconsistency across population subgroups
- **NTDs over time and inclusion of folic acid**

NTD Prevalence



Source: Yen et al. AJDC 1992; 146: 857-61

NTDs and Periconceptional Multivitamin Use - Atlanta

Time Period	Relative Risk
1/68 - 4/72	**0.49**
5/72 - 8/76	**0.32**
9/76 - 12/80	**0.40**

Source: Mulinare et al. JAMA 1988; 260: 3141-5
Erickson (personal communication)

Detracting Evidence or Clues

- Nearly all studies included other vitamins
- Inconsistency across population subgroups
- Prevalence of NTDs over time and inclusion of folic acid
- **Null study findings**

American Journal of Epidemiology

Vol. 168, No. 1
DOI: 10.1093/aje/kwn331
Advance Access publication October 25, 2008

Original Contribution

Neural Tube Defects and Maternal Folate Intake Among Pregnancies Conceived After Folic Acid Fortification in the United States

Bridget S. Mosley, Mario A. Cleves, Anna Maria Siega-Riz, Gary M. Shaw, Mark A. Canfield, D. Kim Waller, Martha M. Werler, and Charlotte A. Hobbs for the National Birth Defects Prevention Study

Initially submitted March 1, 2008; accepted for publication June 11, 2008.

Rates of neural tube defects have decreased since folic acid fortification of the food supply in the United States. The authors' objective was to evaluate the associations between neural tube defects and maternal folic acid intake among pregnancies conceived after fortification. This is a multicenter, case-control study that uses data from the National Birth Defects Prevention Study, 1998–2003. Logistic regression was used to compute crude and adjusted odds ratios between cases and controls assessing maternal periconceptional use of folic acid and intake of dietary folic acid. Among 180 anencephalic cases, 385 spina bifida cases, and 3,963 controls, 21.1%, 25.2%, and 26.1%, respectively, reported periconceptional use of folic acid supplements. Periconceptional supplement use did not reduce the risk of having a pregnancy affected by a neural tube defect. Maternal intake of dietary folate was not significantly associated with neural tube defects. In this study conducted among pregnancies conceived after mandatory folic acid fortification, the authors found little evidence of an association between neural tube defects and maternal folic acid intake. A possible explanation is that folic acid fortification reduced the occurrence of folic acid-sensitive neural tube defects. Further investigation is warranted to possibly identify women who remain at increased risk of preventable neural tube defects.

folic acid; neural tube defects

Abbreviations: B3, 3 months before pregnancy; CI, confidence interval; DFE, dietary folate equivalent; OR, odds ratio; P1, first month of pregnancy.

Editor's note: *An invited commentary on this article appears on page 18, and the authors' response is published on page 22.*

After 3 decades of epidemiologic research reporting an association between neural tube defects and maternal use of folic acid (1–10), public health organizations developed recommendations and supported interventions to increase folic acid intake among women of reproductive age. In 1992, the US Public Health Service recommended that all women of childbearing age who are capable of becoming pregnant should consume 400 μg of folic acid daily (11).

In 1999, the March of Dimes, Centers for Disease Control and Prevention, and National Council on Folic Acid launched the National Folic Acid Educational Campaign. The US Food and Drug Administration had mandated that all enriched cereals and grains contain 140 μg of folic acid per 100 g of grain by January 1998 (12). In 2005, after the National Campaign and mandatory fortification, approximately 33% of women reported taking a daily supplement of folic acid (13), only a modest increase from the 25% reported in 1995 (14). However, median blood folate levels among women of childbearing age increased from 4.8 to 13.0 ng/mL between 1994 and 2000 (15), with a more recent study (16) reporting median blood folate levels at least 2 times the levels prior to fortification.

NTDs & Folic Acid – Recent Data

	Odds Ratios (95% CI)	
	Spina Bifida	Anencephaly
Supplements in month before pregnancy	1.1 (0.9, 1.5)	1.7 (1.2, 2.4)

Mosley et al. Am J Epidemiol 2009.

Folic Acid Intake and Spina Bifida in the Era of Dietary Folic Acid Fortification

Katherine Ahrens, Mahsa M. Yazdy, Allen A. Mitchell, and Martha M. Werler

Background: The US Food and Drug Administration mandated that enriched grain products be fortified with folic acid by 1998. We evaluated whether intake of folic acid from supplements and diet was associated with a reduction in spina bifida in the setting of folic acid fortification.

Methods: Data were collected as part of the Slone Birth Defects Study from 1998 to 2008. Mothers of infants with and without birth defects were interviewed within 6 months of delivery about pregnancy exposures, including details of diet and vitamin intake. Dietary natural folate and synthetic folic acid from fortification were combined into a single, weighted measure—dietary folate equivalent. Periconceptional folic acid supplementation and dietary folate consumption were compared between 205 mothers of spina bifida cases and 6357 mothers of nonmalformed controls. Relative risks of a spina bifida–affected birth were estimated with odds ratios (ORs) and 95% confidence intervals (CIs).

Results: Spina bifida was not associated with regular folic acid supplementation (≥4 days per week) either around the time of conception (adjusted OR = 1.1 [95% CI = 0.74–1.7]) or initiated in early pregnancy (0.79 [0.54–1.2]). After adjustment for confounders, a 13% reduced odds of spina bifida was estimated for each 100-μg increase in daily dietary folate equivalent consumed.

Conclusions: In the setting of folic acid fortification of grains, our data suggest that folic acid supplementation does not appear to offer further benefit for reducing risk of spina bifida. Rather, the folate-associated benefit on spina bifida risk was found with increasing amounts of dietary folic acid consumed, regardless of folic acid supplementation level.

(*Epidemiology* 2011;22: 731–737)

Since 1998, when the US Food and Drug Administration mandated that enriched grain products be fortified with folic acid, the estimated number of pregnancies affected by neural tube defects (NTDs) has declined by approximately 27%.[1,2] However, a greater decline was predicted,[3] raising the question of whether at least some of the remaining cases can be prevented through increased periconceptional supplementation or dietary folic acid intake.

Mosely and colleagues[4] recently investigated this issue, using data collected from the National Birth Defects Prevention Study. The authors found that neither periconceptional folic acid supplementation nor dietary folic acid intake was associated with a reduction in the risk of NTDs, including spina bifida. The objective of our study was to examine risks of spina bifida in relation to maternal folic acid supplementation and dietary intake in another large case-control study, focusing on the time period since folic acid fortification began.

METHODS

The Slone Birth Defects Study was initiated by the Slone Epidemiology Center in 1976. Infants with birth defects were identified by the study staff from discharge records of participating hospitals serving the areas surrounding Boston, MA; Philadelphia, PA; San Diego, CA; and Toronto, Canada; in addition, cases have been identified through birth-defect registries in Massachusetts and parts of New York State. Nonmalformed controls have been randomly selected each month from study hospitals' discharge lists or from statewide birth records. Malformed live-born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation were eligible as cases for our study; however, ascertainment of non–live-born cases has not been routine. Only live-born nonmalformed infants were eligible as controls for our study. The Birth Defects Study has been approved by the institutional review boards of Boston University and relevant participating hospitals and centers.

Mothers of eligible cases and controls were telephoned within 6 months of delivery by a research nurse to conduct the computer-assisted interview. After obtaining informed consent, interviews were conducted in either English or Spanish and lasted for approximately 45–60 minutes.

The interview included questions on the following topics: pregnancy intention, medical and obstetric history, illness and medication history during the period 2 months before the last menstrual period (LMP) through the end of the pregnancy, weight and diet before pregnancy, and behavioral risk factors (such as alcohol consumption and smoking) during pregnancy. In addition, the interview obtained demographic information and family history of birth defects. In the

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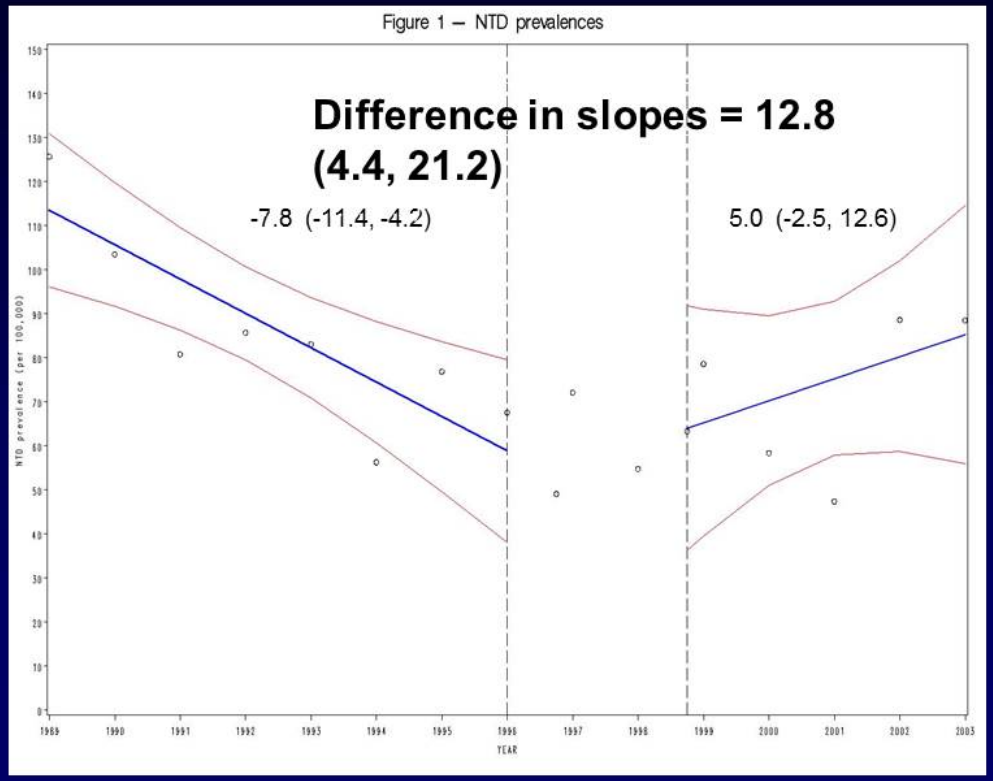
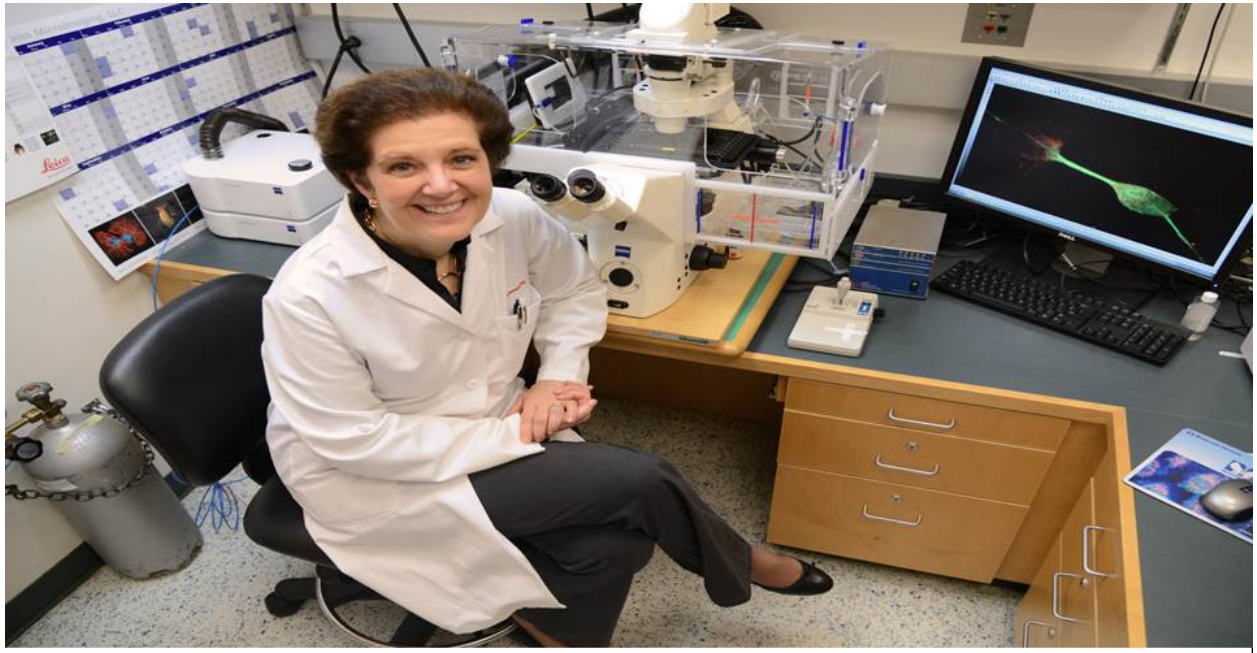
Annual NTD prevalences in central California, 1989-2003





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Effect of Maternal FA Diet on NTD rate in *Lrp6* nulls

Dietary FA	# Observed*		% of *Lrp6–/–* Embryos with NTD	P-value**
	No NTDs	NTDs		
2ppm	7	15	68%	
10 ppm	0	15	100%	0.03


where
do we go
from
here?

Other Nutrients

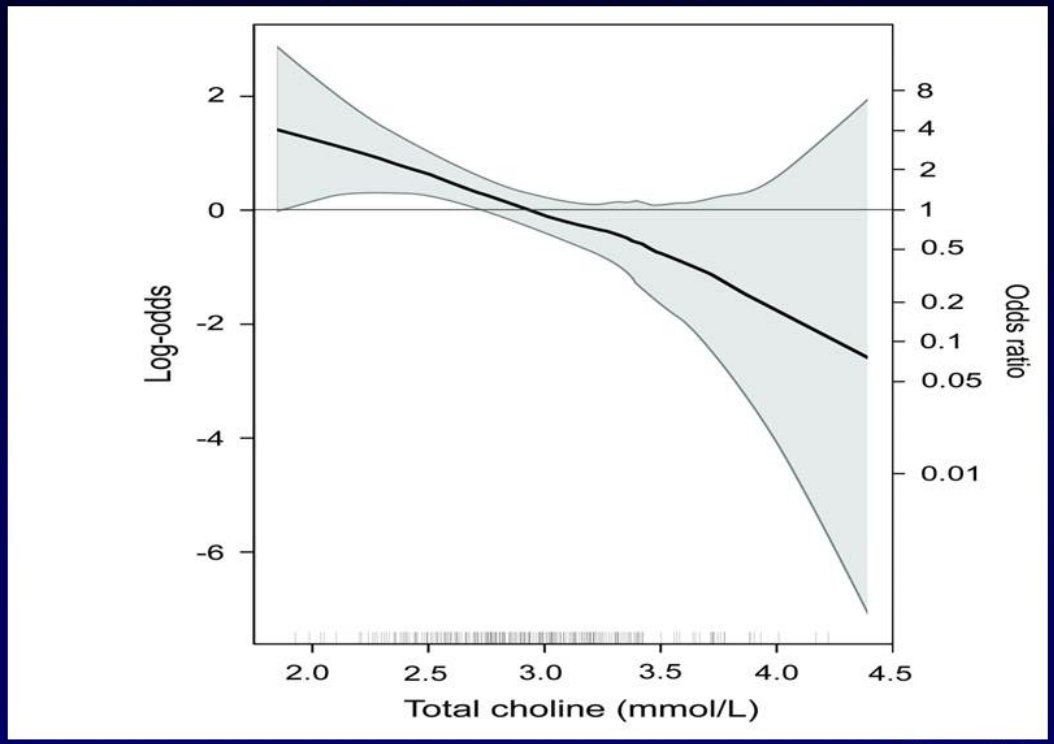
- Vitamin B_{12}
- Methionine
- Zinc
- Inositol
- Choline

NTDs & B_{12}

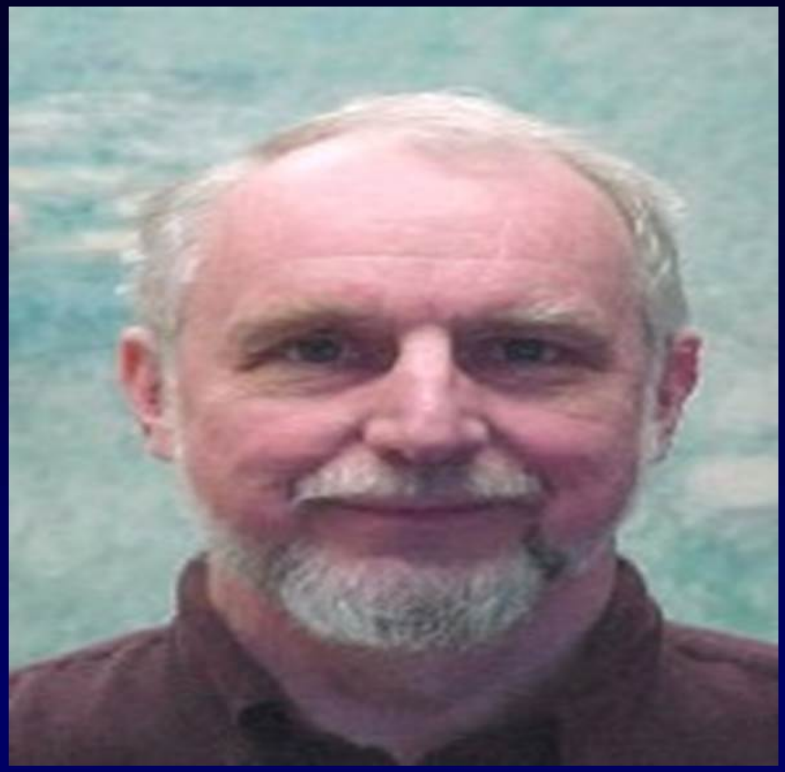
Quartile of B12 serum level	"Group 1"	Odds Ratio (95% CI)
1		3.2 (1.5-6.2)
2		2.8 (1.3-6.0)
3		1.8 (0.8-4.1)
4		Reference

Molloy et al. 2009

NTDs & Serum Choline



Shaw et al.
Epidemiol 2009



Dr. James Mills and colleagues

Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects[1–3]

James L Mills, Ruzong Fan, Lawrence C Brody, Aiyi Liu, Per M Ueland, Yifan Wang, Peadar N Kirke, Barry Shane, and Anne M Molloy

ABSTRACT a beneficial effect of choline could help elucidate the mechanism

"If we knew what we were doing it wouldn't be called research"- Einstein

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PEDIATRIC RESEARCH

Printed in U.S.A.

Nested Case-Control Study of One-Carbon Metabolites in Mid-Pregnancy and Risks of Cleft Lip With and Without Cleft Palate

GARY M. SHAW, STEIN EMIL VOLLSET, SUZAN L. CARMICHAEL, WEI YANG, RICHARD H. FINNELL, HENK BLOM, AND PER M. UELAND

Diet Quality Index

- "Holistic" approach
- Diet quality > single nutrient ?
- 6 (+): grains, vegetables, fruits, folate, iron, calcium
- 2 (–): % calories from fats; sweets

Carmichael et al. 2011

Questions

- Are the folate-sensitive NTDs removed from the population?
- Folic acid works – but how?
  - shed light on development
  - response vary by genetic background?

Questions

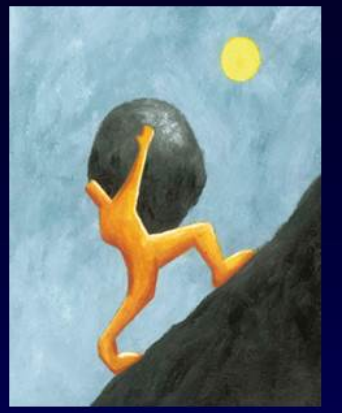
- Are the folate-sensitive NTDs removed from the population?
- Folic acid works – but how?
  - shed light on development
- **Unintended effects:**
  - twinning?
  - Miscarriage ↑?
  -

Questions

- Are the folate-sensitive NTDs removed from the population?
- Folic acid works – but how?
  - shed light on development
- Unintended effects:
  - twinning?
  - miscarriage?
  - birth defects?
  - cancers?
  - childhood asthma?
- Other nutrients are important – but which ones?

“it is better to have an approximate answer to the right question than an exact answer to the wrong one” - Tukey

Collaborators



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